indefinite. The Examiner takes the position that the claim is incomplete because it does not restate the preamble at the end of the claim.

Applicants have amended claim 35 to address the Examiner's objection.

Claims 20-28, 30-36 and 39 are rejected under 35 USC § 102(e) as being anticipated by U. S. Patent No. 5,695,977.

This rejection is respectfully traversed.

The rejected claims actually cover several different embodiments of the present invention. Independent claims 20 and 24 involve the use of a vector having a heterologous expression control sequence/amplification gene and a positive selection marker gene flanked by target sequences for a site-specific recombinase (SSR). This construct is flanked by DNA sequences which are homologous to the target genome to facilitate homologous recombination.

The vector of claim 25 contains a heterologous expression control sequence/amplification gene and a positive selection marker gene flanked by two SSR sites.

Independent claims 28 and 32 involve the use of a vector having a nucleic acid sequence which binds an activator protein (e.g., HIF binder) and a positive selection marker gene flanked by DNA sequences which are homologous to the target genome.

Claim 35 involves the use of a vector having a heterologous expression control sequence which is operatively linked with a reporter gene and non-coding

nucleic acid sequences on the 5'-side and/or the 3'-side from the region of the target gene.

Claim 36 involves the use of a vector having an SSR site which is flanked by DNA sequences which are homologous to a DHFR nucleic acid sequence which is present endogenously in the target cell.

Claim 39 is directed to a vector having a DHFR-encoding sequence, a nucleic acid sequence to be amplified and (optionally) a positive selection marker gene, where this construct is flanked by two SSR sites.

Applicants fail to see how any of the embodiments of the invention discussed above are anticipated by the '977 patent. Applicants have previously noted that the '977 patent is directed to the discovery of "hot spots" for the integration of an exogenous nucleic acid sequence. The inventor in the '977 patent discovered that nicks can be induced at these "hot spots" in a fairly controlled manner. The inventor found that a consensus sequence TTAAAA is susceptible to nicks between the T and A. Thus, with this information in hand, one of ordinary skill in the art could design sequences to be inserted at these sites.

What follows in columns 4-8 is a laundry list of possible elements which can be used in the art of homologous recombination. As previously noted, the '977 patent, *inter alia*, describes the in-out technique (see column 4, line 59 through column 5, line 3) and the use of SSR sites (see column 7, lines 31-43). Of course, these techniques are used in the present invention. However, it is quite clear from

the entire disclosure that the inventor in the '977 patent never contemplated the arrangement of the present invention.

The invention in the '977 patent is used to enhance integration of a construct of interest, where there is little preference for a particular or unique site for integration. See column 4, lines 1-6. The construct of interest has the consensus sequence and may or may not have a second sequence homologous to a sequence in the target region. Joined to the consensus sequence is one or more sequences of interest, which may be coding or non-coding, so as to code for a peptide of interest, an anti-sense sequence, a regulatory sequence, or the like. See column 5, lines 14-39.

The sequence of interest can be in the form of a transcription cassette, which comprises a transcription initiation regulatory region (including a promoter and, optionally, one or more enhancers), the sequence of interest under the transcription regulator, and a transcriptional termination regulatory region (which includes a polyadenylation signal). Connected with the sequence of interest may also be a marker which allows for the selection of hosts containing the construct. See column 6, lines 1-12.

The cited '977 document is concerned with improved efficiency of homologous recombination, using a specific recombination consensus sequence. Applicants agree with the Examiner that the '977 patent mentions the production of recombinant proteins by means of homologous recombination. Contrary to the present invention,

however, an expression cassette is used for this purpose. The expression cassette used the '977 patent contains, as an essential feature, a transcribed sequence of interest (i.e., **an exogenous gene**). There is absolutely no disclosure whatsoever regarding the activation of **endogenous** genes, as claimed herein.

Although the use of recombinase recognition sites is mentioned in the '977 patent (see column 4, lines 22-23 and column 7, lines 31-43), the use of the recombinase recognition sites according to the invention is neither anticipated nor rendered obvious. In the '977 patent, it is only pointed out (for example) that by using recombinase recognition sequences, a variety of sequences may be introduced into a cell to determine the effect of variation in genetic sequence, or to provide cells where one can introduce sequences at a defined site.

There is no absolutely no hint as to the use of the construct described in the present claims, which contain the claimed combinations of elements noted above. Although the '977 patent makes a reference to the insertion of an amplification gene (see column 4, line 25), no hint can be found in this passage as to the combined use of amplification genes and recombinase recognition sequences.

It is not sufficient for the Examiner to cite a reference which merely lists the same elements used in the present invention. Rather, in order to be a valid anticipatory reference, the elements must be arranged as required by the claims. *In re Bond*, 15 USPQ 2nd 1566 (Fed. Cir. 1990). Since the '977 patent neither contains any disclosure whatsoever regarding endogenous gene expression nor

shows the particular arrangement of the vectors used in the present invention, applicants respectfully submit that none of the rejected claims could be considered anticipated by or obvious over the '977 patent.

Claims 20-43 are rejected under 35 USC § 103(a) as being unpatentable over the '977 patent in view of WO 97/37012, WO 94/12650 and Cruz et al. Each of these references was cited in the previous Office Action.

This rejection is respectfully traversed.

WO '012 discloses a genetic construct comprising a recombinase genetic unit (e.g., a *cre* gene) under the control of a first promoter and a transgene unit under the control of a second promoter, wherein the recombinase genetic unit and the transgene unit are linked and flanked by two recombination loci (e.g., *loxP* sites). Optionally, the genetic construct can further comprise left border and right border sequences to facilitate its *in vivo* insertion into chromosomal DNA. The genetic construct is shown, for example, in Fig. 5.

WO '012 neither includes a reference to endogenous gene activation nor to homologous recombination. The reference only describes the use of an expression cassette containing a transgene in combination with a sequence coding for a recombinase, flanked by two recombination sequences. This construct is used for transfecting cells, especially plant cells. There is no hint whatsoever as to homologous recombination.

Like the present invention, WO '650 relates to endogenous gene expression.

However, there is absolutely no reference whatsoever to the use of site-specific recombinase recognition sequences and the use of the claimed constructs, respectively, which are flanked by recombinase recognition sequences. One of skill in the art would never had considered combining WO '650 and WO '012 as of the priority date of the present application, since the two documents each relate to totally different technical fields (heterologous gene expression on the one hand and endogenous gene activation on the other hand).

The Cruz publication describes the inactivation of a DHFR gene in a protozoal cell. There is no reference whatsoever to site-specific recombinase and the recognition sequences thereof, respectively. Rather, this publication is merely concerned with the analysis of the gene function in protozoa and includes no reference whatsoever to the preparation of recombinant proteins.

In the Discussion section of Cruz, it is pointed out that the method described can be applied only in the case of protozoa. Thus, to make it clear that Cruz has absolutely nothing to do with the present invention, applicants have amended claims 36, 37 and 41-43 to restrict the type of cell used in these embodiments of the invention to a mammalian cell. Applicants note that the vectors according to claims 39 and 40 are novel and inventive in view of Cruz, since no reference can be found therein as to constructs having recombinase recognition sequences.

In summary, applicants respectfully submit that the Examiner is combining the cited references in an inadmissable hindsight approach, which is possible only when

knowing the present invention. The Examiner has arbitrarily picked out features from individual citations and has taken them out of their context to combine them with features also taken arbitrarily from other references. Therefore, applicants respectfully submit that the rejections are unfounded, and should be withdrawn.

In the event this paper is not timely filed, applicants hereby petition for an appropriate extension of time. The fee for this extension may be charged to our Deposit Account No. 01-2300, along with any other additional fees which may be required with respect to this paper.

Respectfully submitted,
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